PTO 08-0530

CC=DE
DATE= 19920527
KIND= OLS
PN= 4037447

PHENYLALANINE-FREE DIET FOR JUVENILES AND ADULTS AFFLICTED WITH PHENYLKETONURIA
[PHENYLALANINFREIES DIÄTETIKUM FÜR JUVENILE UND ADULTE PERSONEN MIT PHENYLKETONURIE]

WACHTEL URSULA, SCHWEIKHARDT FRIEDRICH, TESMER ERHARD

UNITED STATES PATENT AND TRADEMARK OFFICE
WASHINGTON, D.C. OCTOBER 2007
TRANSLATED BY SCHREIBER TRANSLATION, INC.

PUBLICATION COUNTRY	(10):	Germany
DOCUMENT NUMBER	(11):	4037447
DOCUMENT KIND	(12):	Patent specification laid open to inspection
PUBLICATION DATE	(43):	19920527
APPLICATION NUMBER	(21):	P 40 37 447.5
APPLICATION DATE	(22):	19901124
INTERNATIONAL CLASSIFICATION	(51):	A 23 L 1/305, A 23 L 1/09, A 23 L 1/304, A 23 L 1/302, //A 61K 31/195
PRIORITY COUNTRY	(33):	
PRIORITY NUMBER	(31):	
PRIORITY DATE	(32):	
INVENTOR(S)	(72):	Wachtel Ursula Dr., 6380 Bad Hamburg, Germany; Schweikhardt, Friedrich Dr., 6382 Friedrichsdorf, Germany; Tesmer, Erhard Dr., 6380 Bad Hamburg, Germany
APPLICANT(S)	(71):	Milupa AG, 6382 Friedrichsdorf, Germany
DESIGNATED CONTRACTING STATES	(81):	
TITLE	(54):	PHENYLALANINE-FREE DIET FOR JUVENILES AND ADULTS AFFLICTED WITH PHENYLKETONURIA
FOREIGN TITLE	[54A]:	PHENYLALANINFREIES DIÄTETIKUM FÜR JUVENILE UND ADULTE PERSONEN MIT PHENYLKETONURIE

/1

Phenylalanine-free diet for juveniles and adults afflicted with phenylketonuria

The inventive phenylalanine-free diet based on amino-acids for juveniles and adults afflicted with phenylketonuria contains amino-acids of at least 95% and preferably, exclusively the following amino-acids namely L-histidine, L-isoleucine, L-Valine, L-treonine, L-methionine, L-leucine, L-Tryptophane, L-tyrosine and L-Lysine. The sustenance requirement of proteins of adolescent and grown persons afflicted with phenylketonuria can be covered with this diet.

In the inventive process for manufacture of phenylalanine-free diet, the amino acids used and the other components which may be present are together dissolved or dispersed in water and finally, spray-dried. The powder obtained in this way cannot be separated and is free-flowing.

---

[1]Numbers in the margin indicate the pagination in the foreign text.

Description

The invention relates to a phenylalanine-free diet based on amino acids for persons, especially juveniles and adults who are afflicted with phenylketonuria, and also a process for the manufacture of phenylalanine-free diet.

Phenylketonuria belongs to the group of genetic disorders that produce deficiencies in which changed, coded sequences of deoxyribonucleic acid determine the state of the disease. The consequence of gene mutation leads to expression of abnormal proteins. Therefore, the pathogenesis of enzymopathy, i.e. those diseases that are caused due to reduced or missing activity of an enzyme, is based on the accumulation of the unconverted substrates during metabolism and their metabolites.

Phenylalanine is absorbed with all proteins of animal and plant origin under normal diet conditions. After digestion of native proteins and reabsorption of fission products, phenylalanine reaches the liver via the amino acids- pool and there, it is hydroxylated into tyrosine in an irreversible reaction by the enzyme phenylalanine hydroxylase. In case of babies and small children, a part of phenylalanine is required for the body's own protein synthesis subsequent to rapid growth.

At present, eleven different mutations in the phenylalanine hydroxylase- gene are known. If the mutation leads to complete deficiency of activities of this enzyme, then, a so-called "Phenylketonuria" (subsequently abbreviated as PKU) occurs. If the enzyme has only

reduced activity, then, this leads to so-called "Hyperphenylalaninemia" which must be treated partially.

As a consequence of metabolism disturbance, phenylalanine accumulates in the body for the sick persons or patients by which its content in blood and in tissues increases way above the normal range. The phenylalanine then breaks down via the byways of metabolism with increasing concentration; these byways are generally not used by the body.

The increased phenylalanine level in plasma leads to impairment of numerous metabolism processes in the brain. If PKU is not treated, it could lead to cerebral maturation disorder with mental retardation in varying forms.

The causes of toxic effect of increased phenylalanine level in plasma on the cerebral metabolism appear to be based on disturbance of myelinization. Even deficiency of catecholamine and serotonin as well as increased turnover of phenyl ethylamine appears to play a role.

Till recently, it was assumed that increased phenylalanine level can cause disturbance in the cerebral metabolism only till the beginning of puberty. Newer trials in young children for whom treatment was stopped in the school-going age, showed that it led to neurological disturbances; for e.g. distinctly measurable decline of reaction time period was observed.

The severity code of neurological disturbances is different for individual patients. Thus, we can conclude that myelinization of brain does not end with the beginning of puberty as opposed to the earlier concept, but it is a constant, on-going metabolism process.

Phenylketonuria can be treated more or less successfully with phenylalanine-poor diet.

In order to attain normal mental and physical growth for a child suffering from PKU, its phenylalanine level in the plasma must be reduced to normal values with a phenylalanine-poor diet and the phenylalanine level must be stabilized.

For this, children are given a diet that contains restricted quantity of natural protein and that much quantity of phenylalanine which is required by the body for building up of protein (growth). For the nourishment of the child, only such foodstuffs can be used that have low protein content by nature and thus, have low content of phenylalanine.

But, with such a type of phenylalanine-poor diet alone, the child will be given too less of all other amino acids that are essential for existence. Therefore, children suffering from PKU, require, in addition to a phenylalanine-poor diet, a protein source which does not contain phenylalanine, but sufficient quantity of all other amino acids.

Already, specialty products are known that are made up of protein blocks, the amino acids and do not contain any phenylalanine. These mixtures are assimilated in addition to

vitamins, mineral nutrients and trace elements since a child suffering from PKU will not get these nutrients in sufficient quantities with phenylalanine-poor nutrition.

Due to the tendency of cerebral metabolism to develop due to increased phenylalanine level in plasma, even after attaining puberty, experts advise their patients to maintain phenylalanine-poor diet for life.

The human problems arising especially from the compulsion of retaining such a diet should not be overlooked because of the medicinal aspects of care of children suffering chronically from PKU.

A difficult age in this respect is especially the adolescence in which the treatment requirement of PKU is often refused by the patient and is neglected. It has been shown that the lacking adherence of diet or its refusal adversely affects the development of patients; how much ever lower the phenylalanine level , so much more favorable the intellectual, neurological and psychomotor development over the time period of treatment.

The foodstuffs that are used as protein source contain proteins which are made up of 20 different amino acids. The proteins and therefore, the amino acids that synthesize these proteins must be supplemented constantly with the nourishment since retention is possible only in a very limited scope.

From the nutritional point of view, amino acids are classified into essential (indispensable), semi-essential and non-essential amino acids. The essential amino acids which the human body cannot synthesize are as follows: Threonine, leucine, phenylalanine, methionine, isoleucine, tryptophan, valine and lysine. Arginine and histidine are considered as semi-essential which must be fed only in the growth phases or in case of deficiencies. Cystine and tyrosine assume an exceptional position since they must be synthesized in the body in case the exogenous supply is not sufficient.

/3

So far, the concept was that the protein metabolism requires simultaneous presence of all and even the non-essential amino acids. Due to this reason, the known and usually used specialty products or phenylalanine-free amino acid mixtures for babies and children with high need for growth contain all amino acids with the exception of phenylalanine which disturbs its metabolism.

Depending on the individual metabolism situation of a patient suffering from PKU, he must take 45 to 70 g of known phenylalanine-free amino acid mixture per day. This is a considerable amount of substance. The acceptance of these known specialty products by patients can have very strong negative effects, especially for juveniles.

The task of this invention is, therefore, to prepare a phenylalanine-free diet or a phenylalanine-free amino acid mixture which is better accepted by patients suffering from PKU and conforms to the requirements of life-long intake of diet.

This problem is solved by the phenylalanine-free diet according to Claim 1.

It was surprisingly found that the daily protein requirement of juveniles and adults suffering from PKU can be covered completely or almost completely with amino acid mixture which contains predominantly or exclusively only essential amino acids (compare the comments given above) with the exception of phenylalanine as well as L-

tyrosine and L-histidine. In case of these patients, the growth requirement is not met by intake of these proteins. The sustenance requirement of body substance must still be covered entirely.

Therefore, the inventive diet contains only specific amino acids of at least 95%. Even such diets can be considered as inventive diets which contain only low quantities of other amino acids; but, are not counted as amino acids listed expressis verbis in Claim 1. Preferably, only these specific or nominally listed amino acids are exclusively available.

The amino acids used as per the invention exist naturally in the L-form, even if this is not always specified.

The amino acids are used preferably as free amino acids up to lysine. Lysine exists preferably as lysine acetate. The amino acids can exist in any suitable form and especially in any of the forms permissible for foodstuffs. These amino acids can exist for e.g. as salts, hydrochlorides, hydrates, glutamates, acetates, asparates, glutamates, malates etc.

Since the weight and quantity of the inventive diet must be as less as possible, the amino acids are preferably used – as mentioned above- as free amino acids. Even oligopeptides which are synthesized predominantly or exclusively from the inventive amino acids, can be used as long as they are free from phenylalanine.

The inventive diet covers primarily for juveniles (starting from older school-children) and adults; it can be used even for children as long as the other amino acids required for growth are covered.

If patients suffering from PKU use the inventive diet, they have the advantage that they need to consume only half the daily dosage when compared to the so far known amino acid mixture. This can lead to better "compliance" of patients and a more favorable intellectual, neurological and psychomotor development.

A fine-meshed follow-up of a total of 10 juveniles and adults suffering from PKU was undertaken for several years. It showed that the daily protein requirement can be covered by the inventive phenylalanine-free mixture of certain amino acids along with small quantities of natural protein.

Even for a protein supply of 0.65 g per kg of body weight and day, there is no clinical indication of catabolism. This quantity lies distinctly below the standard values recommended for juveniles and adults, namely 0.9 per kg of body weight and day

according to World Health Organization (1985) or 45 to 50 g per day according to the German Organization for nutrition (1989).

Each patient must take in a daily quantity of 8 to 22 g of inventive phenylalanine-free diet based on certain amino acids depending on his individual metabolism situation compared to 45 to 70 g of amino acid mixture known so far. The result of this extraordinary simplification of diet was that none of the patients gave up the treatment.

According to a preferred embodiment, the inventive diet consists of sugar, mineral nutrients, trace elements and/or vitamins as well as if necessary, monoglycerides and/or diglycerides along with amino acids.

The amino acids are available preferably in the following proportions, expressed in weight -% and expressed as free acids and also with respect to the total of free amino acids:

Amino acid	Weight-%
L-Lysine	14.5
L-Histidine	5.0
L-Isoleucine	11.9
L- Leucine	20.2
L- Methionine	4.9
L-Threonine	9.5
L- Valine	14.4
L- Tryptophan	3.6
L- Tyrosine	16.0
	100.0

The proportions of used amino acids are calculated and adjusted with one another in such a way that the mixture is optimum as far as possible for the covering of sustenance requirement.

/4

These proportions of individual amino acids can vary depending on the individual metabolism situation. Thus, the proportions of lysine, isoleucine, leucine, valine and tyrosine can deviate by up to ± 10% and the proportions for the remaining amino acids by up to ± 20% from the mentioned value. If one (or many) amino acid is used in a large quantity, then, it is obvious that one (or many) other amino acid is used in a smaller proportion.

Further, the proportions or contents of tryptophan and tyrosine in the inventive mixture can be chosen depending on the individual neurotransmitter synthesis.

The daily dosage of the inventive diet required for a patient is divided appropriately into small portions of 5 to 10 g whereby up to three such portions are taken per day. The consumption of such small volume leads to better acceptance by the patients. The regular consumption distributed evenly throughout the day of such portions leads to anabolism in protein metabolism and also to well-balanced amino acid level in the blood due to which imbalances are avoided.

During the manufacture of amino acid mixtures, it has been shown that these can be separated easily, especially, if the amino acids are mixed dry in fine-crystalline, grounded form. Amino acid mixtures that were added to sugar additives showed tendency towards browning and cannot be preserved well.

As per the invention, even a process for the manufacture of phenylalanine-free diet is prepared based on amino acids. This process is not limited to the manufacture of inventive diet containing only certain amino acids, but it can be used even for amino acid mixtures that contain other amino acids.

It was surprisingly shown that the inventive amino acid mixture which can contain even mineral nutrients, sugar, trace elements, vitamins and monoglycerides or diglycerides, is very stable and does not segregate if the components of this mixture are dissolved together in water and finally, spray-dried. Such a product can be dosed well in a free-flowing manner and can be preserved for 2 to 3 years in doses exposed to N_2/ CO_2 or in vacuum packs. The product can be very well preserved even in aluminum compound foils.

To avoid browning reactions, the actual components of phenylalanine-free diet is dissolved in cold water. For this purpose, all mineral nutrients are firstly dissolved. During the dissolution of individual amino acids, a certain sequence of addition of individual amino acids is preferably maintained.

The pH-value of the obtained preparation is controlled in such a way that this pH-value does not drop too much and preferably, not below 5.5 to 5.6. Preferably, the pH- value is set to 6.0 to 6.2 with preferably potassium hydroxide or potassium carbonate, such that the pH maintains this value. In this pH-range, the preparation remains stable.

After dissolution or dispersion of all amino acids and mineral nutrients, the preparation is heated, preferably to about 70 to 80°C. Subsequently, the remaining components of phenylalanine-free diet are added according to the details in Example 1. Preferably, even monoglycerides and/or diglycerides are added which have proved to be very favorable as coating substances and lubricants.

After addition of all components, the obtained preparation is homogenized by which a very intensive and fine-grained distribution of all components is brought about. This contributes also to the fact that no separation of components takes place anymore after the final spray drying.

Due to the addition of monoglycerides and/or diglycerides, the wettability of the obtained powder is further improved by dissolution in water. The powder mixture forms a homogeneous dispersion in water.

The inventive method leads to a very homogeneous distribution of all components. A separation, even during storage and transportation does not take place. The inventive diet is fully homogeneous, can be wetted well and can be dispersed in water and can also be

well-preserved. The Maillard- reaction or browning is minimized. The product obtained is free-flowing and can be dosed.

Example 1

Composition of an inventive phenylalanine -free diet

Monoglyceride	1.00%
Sugar	1.85%
Vanilline	0.05%
L-Lysine acetate	15.20%
L-Histidine	3.70%
L-Isoleucine	8.80%
L-Leucine	14.90%
L-Methionine	3.60%
L-Threonine	7.00%
L-Tryptophan	2.70%
L-Valine	10.70%
L-Tyrosine	11.80%
Mineral nutrient mixture	18.00%
Vitamin mixture	0.70%
	100.00%

Manufacture of a batch of 100 kg of inventive phenylalanine-free diet

150 l of cold tap water is taken in a heatable, jacketed tank provided with a Y-beam stirrer. The following components are added to the cold water one after another with the help of a venturi -- nozzle:

18.0 kg of mineral nutrient mixture
3.7 kg of L-histidine
8.8 kg of L-Isoleucine
14.9 kg of L-Leucine
15.2 kg of L-Lysine acetate
3.6 kg of L-Methionine

7.0 kg of L-Threonine
2.7 kg of L-Tryptophan
10.7 kg of L- Valine
11.8 kg of L-Tyrosine
1.85 kg of sugar

While adding the individual components with the help of a venturi- nozzle, the preparation is stirred simultaneously with the Y-beam stirrer strongly till a homogeneous, lump-free dispersion is obtained.

Subsequently, the preparation is heated to 70 to 75°C, 1 kg of monoglyceride is added and distributed homogeneously. After that, 50 g of vanilline and 700 g of vitamin mixture is dissolved in the preparation.

After that, the preparation is heated for about 15 minutes at 75 to 80°C. Finally, a single-stage homogenization is undertaken at 100 bar. The obtained concentrate with 40% TS (dry substance) is dried into a powder on a nozzle spray tower. The inlet temperature of tower is 180 to 185°C; the outlet temperature of tower is 85 to 90°C.

The obtained powder can be packed into portions each of 8 g in aluminized bags.

Patent claims

1. Phenylalanine-free diet based on amino acids for persons afflicted with phenylketonuria, especially for juveniles and adults, **characterized in that** the amino acids constitute at least 95% and preferably, exclusively the following amino-acids namely L-histidine, L-isoleucine, L-Valine, L-threonine, L-methionine, L-leucine, L-Trypthophane, L-tyrosine and L-Lysine.
2. Phenylalanine-free diet according to Claim1, characterized in that it contains amino acids that are exclusively L-histidine, L-isoleucine, L-Valine, L-threonine, L-methionine, L-leucine, L-Trypthophane, L-tyrosine and L-Lysine.
3. Phenylalanine-free diet according to Claim 1 or 2, characterized in that it also contains sugar, mineral nutrients, trace elements and/or vitamins and if necessary, monoglycerides and/or diglycerides.

4. Phenylalanine-free diet according to Claim 2 or 3, characterized in that it contains the following amino acids in the following proportions, expressed as free amino acids in weight- % and with respect to the total of amino acids, whereby L-lysine exists especially as L-Lysine acetate, while the remaining amino acids are available especially as free acids.

Amino acid	Weight-%
L-Lysine	14.5 ± 10%
L-Histidine	5.0 ± 20%
L-Isoleucine	11.9 ±10%
L- Leucine	20.2 ± 10%
L- Methionine	4.9 ± 20%
L-Threonine	9.5 ± 20%
L- Valine	14.4 ± 10%
L- Tryptophan	3.6 ± 20%
L- Tyrosine	16.0 ± 10%
	100.0

5. Phenylalanine-free diet according to at least one of the Claims 1-4, obtained in that the used amino acids and if necessary, available mineral nutrients, sugar, trace elements, vitamins and monoglycerides/diglycerides are dissolved or dispersed in water and then, spray-dried.

6. Phenylalanine-free diet according to Claim 5, obtained in that the mineral nutrients and the amino acids and if necessary, sugar are dissolved or dispersed in cold water, especially of 3 to 10°C, then the preparation is heated, especially to 70- 80°C and finally the remaining components are added.

7. Phenylalanine-free diet according to Claim 5 or 6, obtained in that the following amino acids are added in the following sequence: L-Histidine, L- isoleucine, L-Leucine, L-Lysine, L-Methionine, L-Threonine, L-Tryptophan, L-Valine, L-Tyrosine.

8. Phenylalanine-free diet according to at least one of the claims 5-7, obtained in that the preparation which is obtained after dissolution of all components is homogenized.

9. Phenylalanine-free diet according to at least one of the claims 5 -8, obtained in that the pH-value of the preparation is not allowed to sink below 5.5 and is maintained especially at 6.0 - 6.2, especially by adding potassium hydroxide or potassium carbonate.

10. Process for manufacture of phenylalanine-free diet based on amino acids, characterized in that the amino acids and if necessary, mineral nutrients, sugar, trace elements, vitamins and/or monoglycerides/ diglycerides are dissolved or dispersed in water and then, spray-dried.

11. Process according to Claim 10, characterized in that the mineral nutrients and the amino acids and if necessary, sugar are dissolved or dispersed in cold water, especially having a temperature of 3 to 10°C, then, the preparation is heated, especially to 70-80°C, and finally, the remaining components are added.

12. Process according to Claim 10 or 11, characterized in that the following L-amino acids are used or added exclusively in the following sequence: L-Histidine, L-Isoleucine, L-Leucine, L-Lysine, L-Methionine, L-threonine, L-Tryptophan, L-Valine, L-tyrosine.

13. Process according to at least one of the claims 10 -12, characterized in that the preparation obtained after dissolution of all components is homogenized.

14. Process according to at least one of the claims 10 - 13, characterized in that, the pH- value of the preparation is not allowed to sink below 5.5 and maintained especially at 6.0- 6.2, especially by adding potassium hydroxide or potassium carbonate.

- Blank page -